Bioburden Control, Cleaning and Disinfection

STERIS
Life Sciences

NASA Conference

March 25, 2015

Jim Polarine MA.
Larry Zanko

Science & Solutions for Life

Agenda

- Bioburden in Cleanrooms
  - Operator Contamination
  - Fungal Spore Contamination
  - Bacterial Spore Contamination
- Cleaning and Disinfection
- *In situ* testing case study

Review - Microflora in Cleanrooms (U.K.)

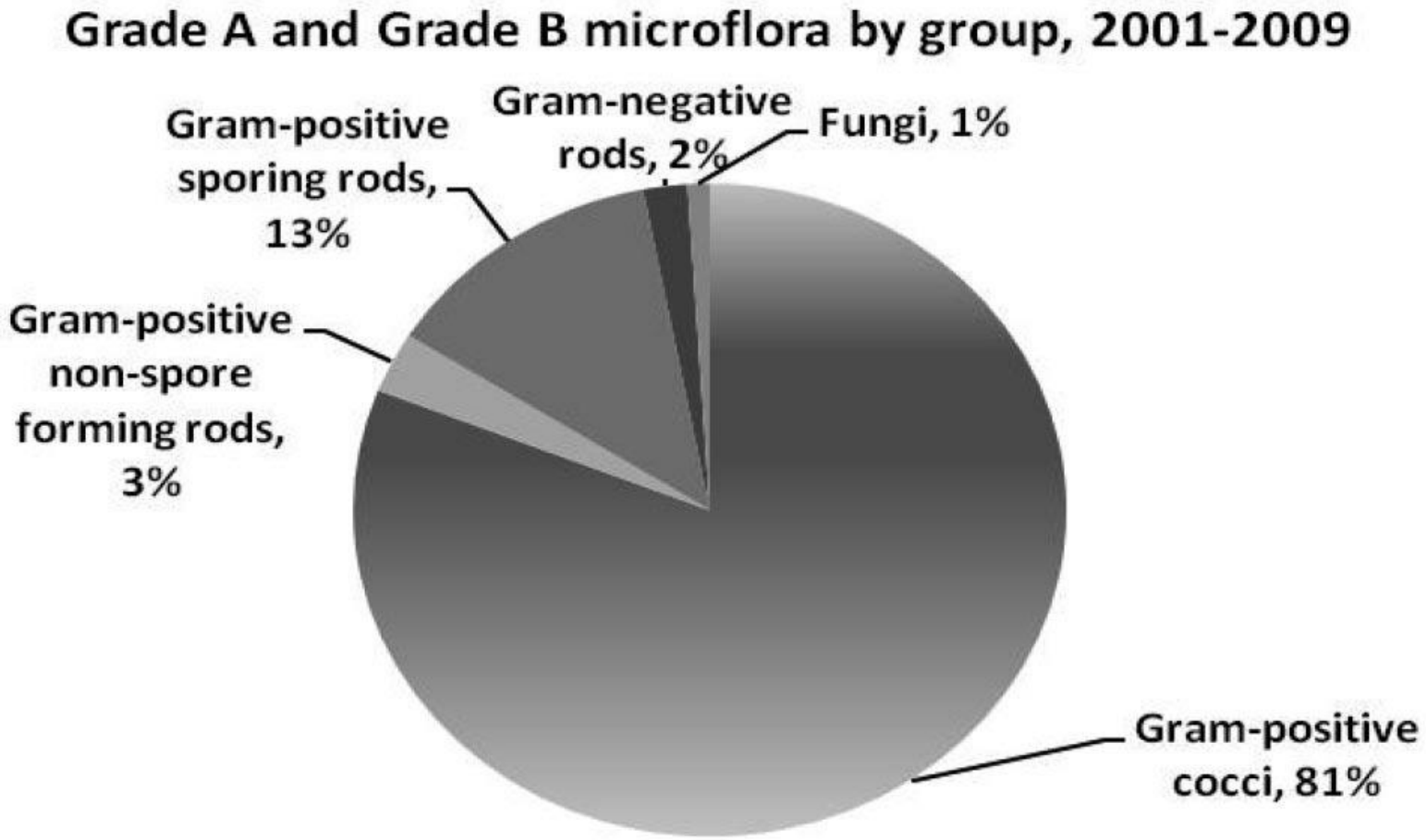
STERIS
Life Sciences

- Tim Sandle
- PDA J Pharm Sci and Tech 2011, 65:392-403
- **A Review of Cleanroom Microflora: Types, Trends, and Patterns**

- Examined isolates from 2000-2009 in U.K.
- Grade A/B and C/D

Review - Microflora in Cleanrooms (U.K.)

STERIS
Life Sciences



Review - Microflora in Cleanrooms (U.K.)

STERIS
Life Sciences

Genus	A/B (6729)	C/D (2500)
Micrococci (and related)	38%	40%
Staphylococci	21%	11%
Bacillus (and related)	13%	10%
Pseudomonas (and related)	<1%	8%
Corynebacterium (and related)	3%	5%
Rhodococci	<1%	N/A
Fungi	N/A	3%

Operator contamination

- *Staphylococcus*
- *Propionibacterium acnes*

Agenda

- ✓ Operator Contamination
- ✓ Fungal Spore Contamination
- Bacterial Spore Contamination

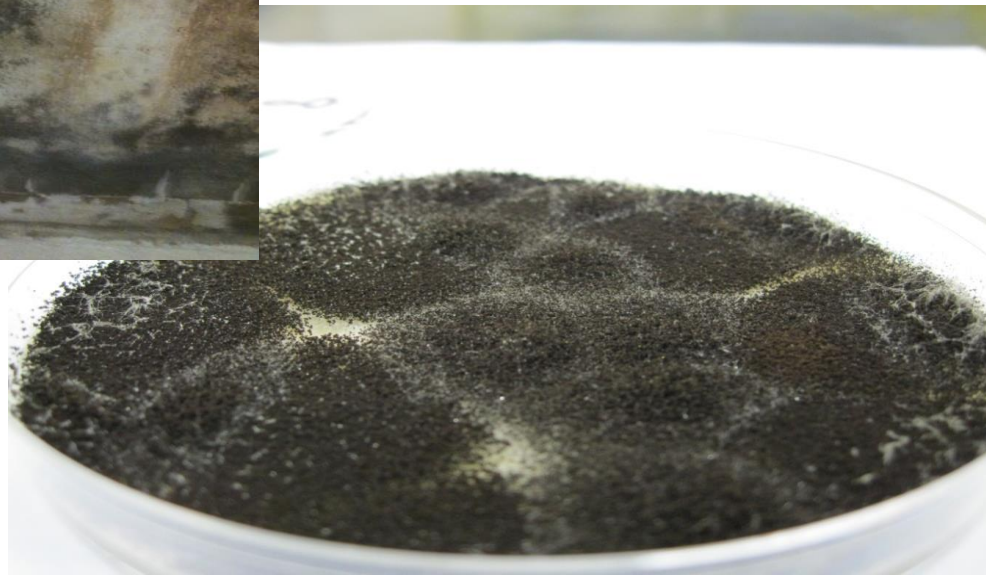
Fungal Spores

- *Penicillium*
- *Aspergillus*
- *Cladosporium*

Penicillium, photos: Ann Larson

Cleanroom Fungi

Courtesy Dan Klein



Common sources of Spores

- Items brought into the Cleanroom
  - Bags, Boxes, Intervention Equipment, Pallets, Pallet Jacks, Scrubbers, Cart Wheels, Shoes, Shoe Covers
  - Raw Materials

Penicillium

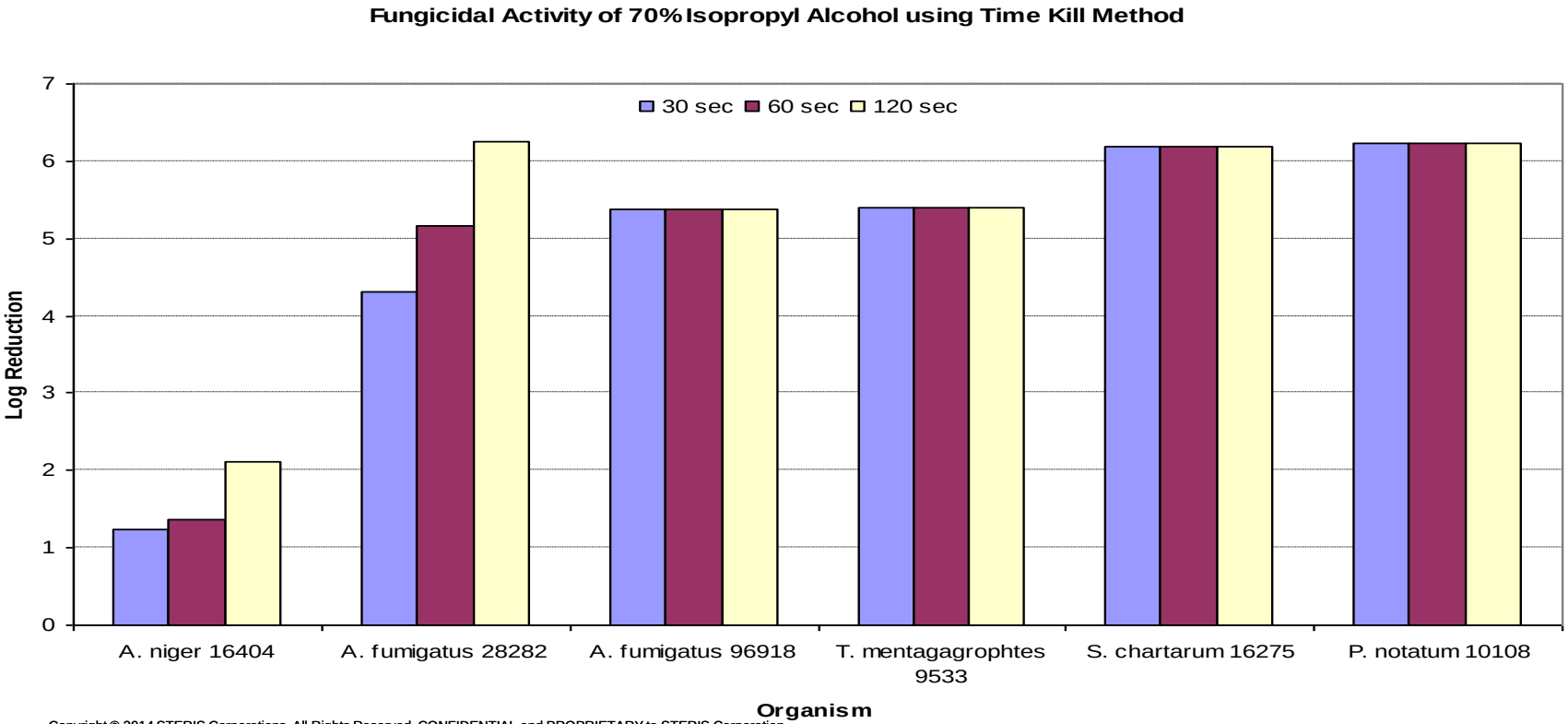
- ISO-7 Cleanrooms
- Action Levels of 10 and picking up >100
  - Engineering Investigating
  - HVAC
  - Duct Work
  - HEPA Filters
  - Cooling Coils
  - Wall Coverings
  - Airflow Vents

Penicillium Investigation

- Entry and Exit Procedures
- Gowning Procedure
- Cart Wheels
- Construction
  - Further Investigation
  - Use of Sporicides
  - Containers in the Cleanroom
  - Cold room Cleaning Procedures
  - Documentation
  - Assignable Cause

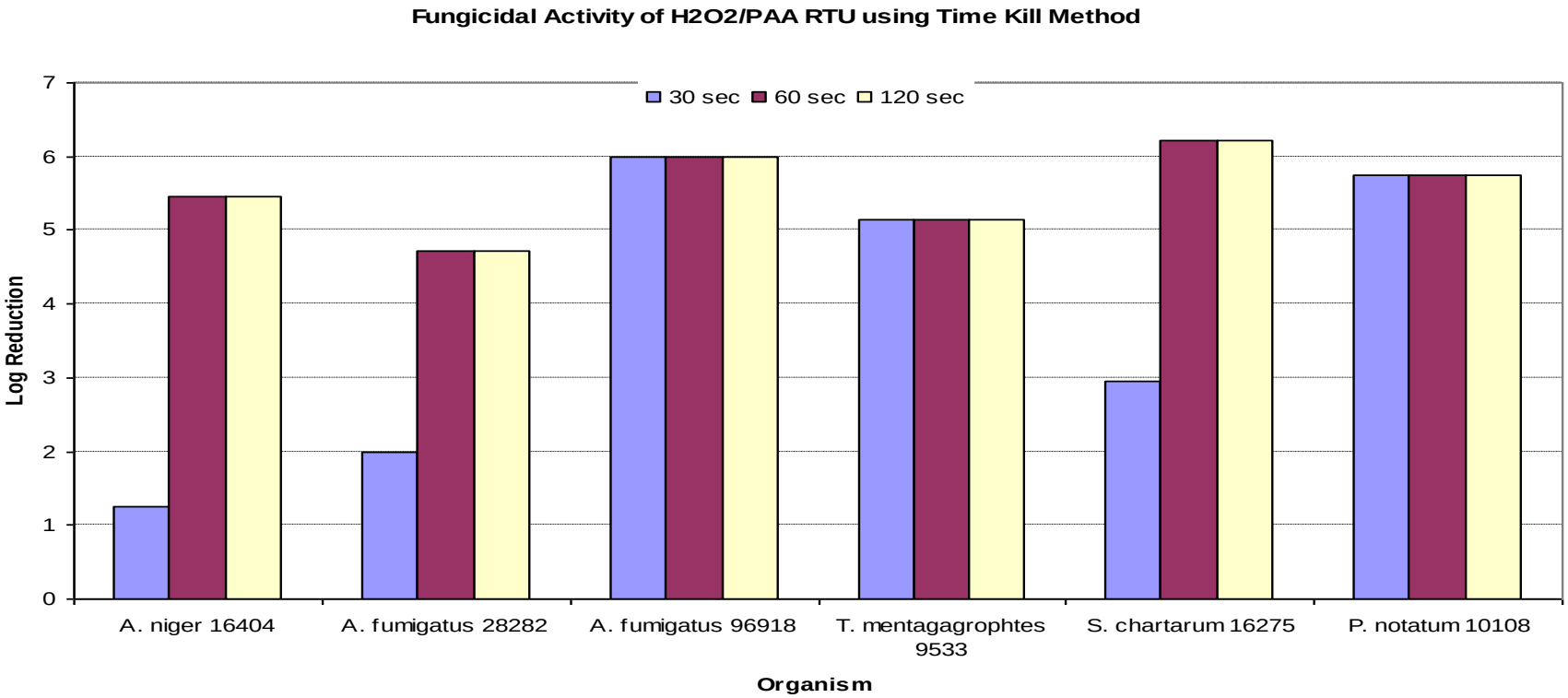
70% IPA Efficacy Against Molds

STERIS
Life Sciences



H_2O_2/PAA RTU Against Molds - Suspension

STERIS Life Sciences



Agenda

- Bioburden in Cleanrooms
  - Operator Contamination
  - Fungal Spore Contamination
  - Bacterial Spore Contamination
- Bacterial Spore Morphology and Efficacy Testing
- *In situ* testing example

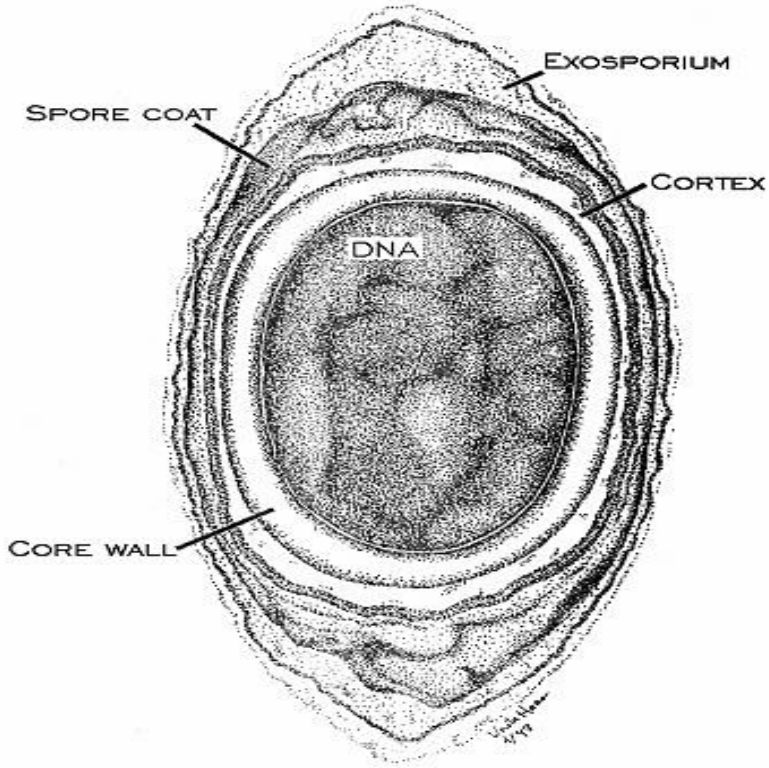
Bacterial Spores

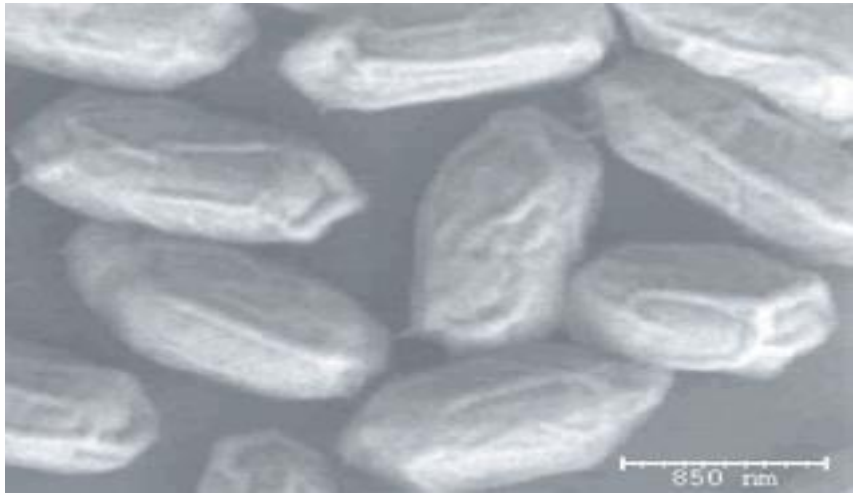
- *Bacillus cereus* group (7 species*)
- *Bacillus circulans*
- *Paenibacillus glucanolyticus*

B. anthracis, B. cereus, B. pseudomycoides, B. mycoides, B. thuringiensis,
B. weihenstephanensis, B. manliponensis

Bacterial Endospore

STERIS
Life Sciences

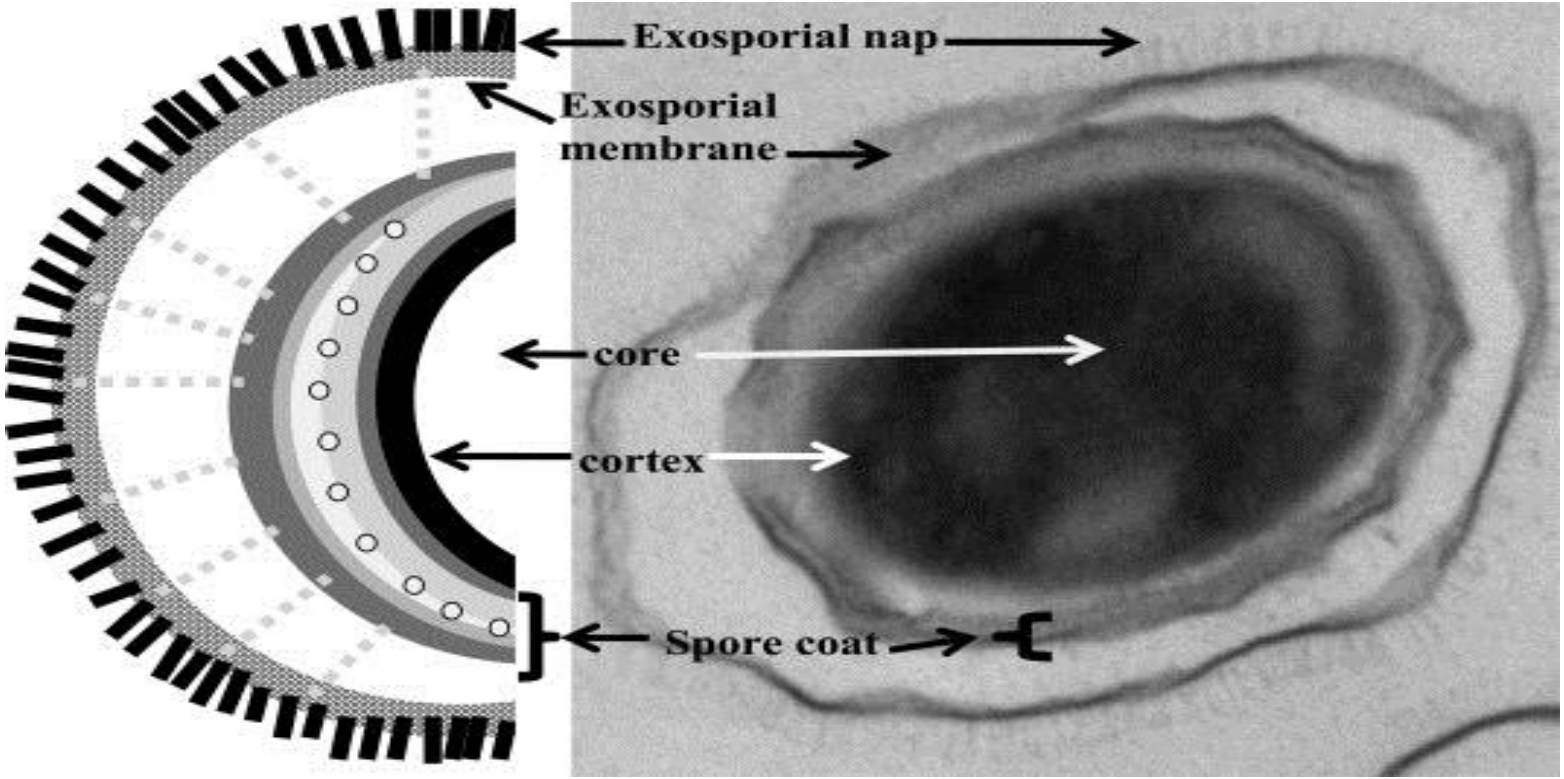




Courtesy Dan Klein

Exosporium – *B. anthracis*

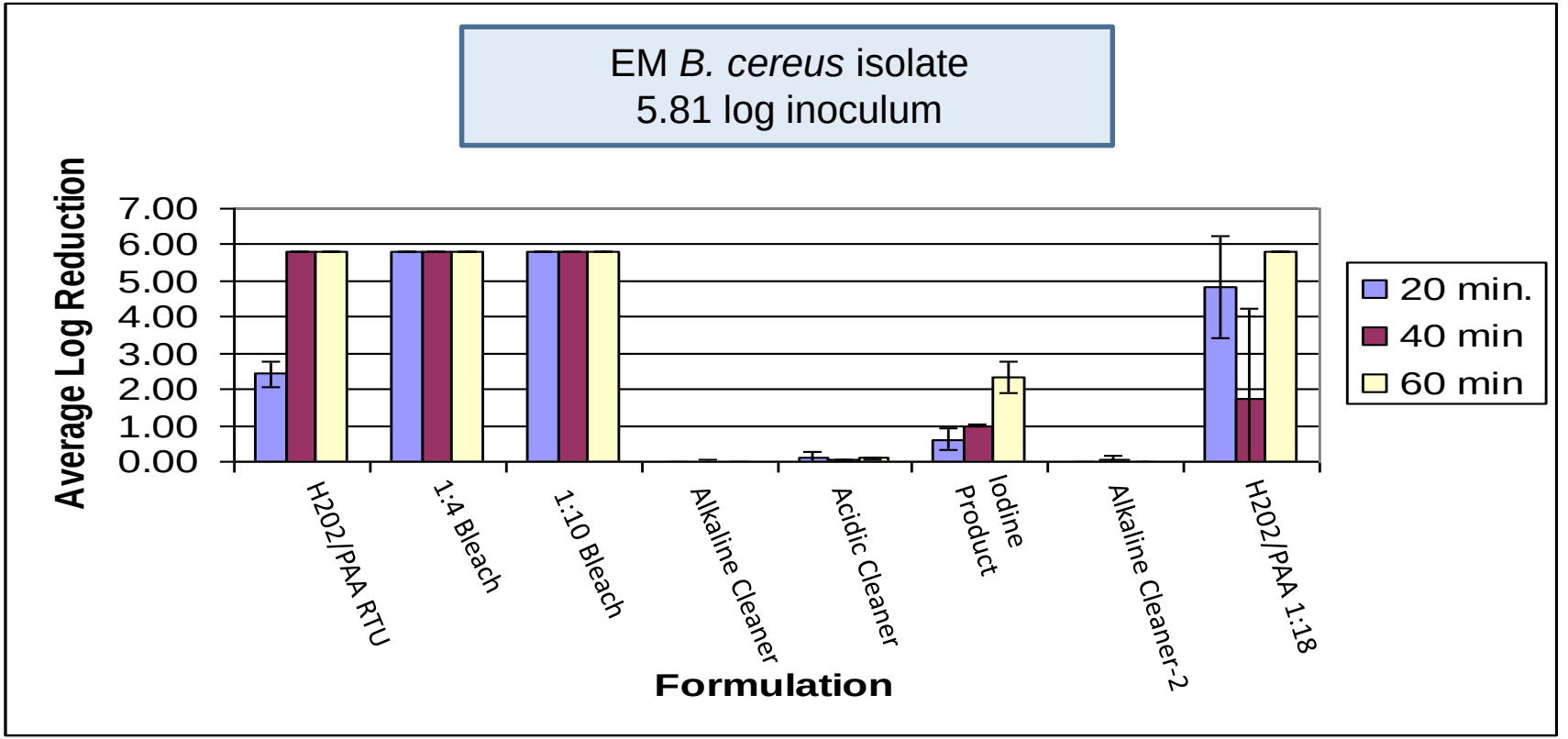
Cote CK et al. 2011. Microbes and Infection 13(14-15):1146-55.



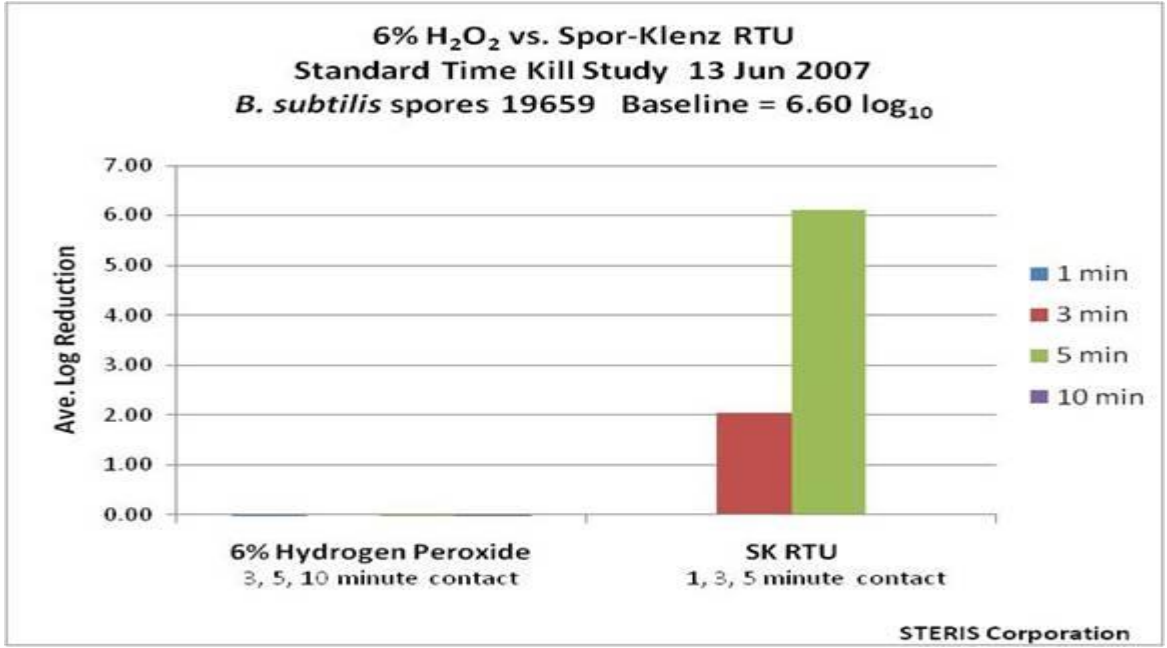
Bacillus cereus

- ISO-7 and ISO-8 cleanrooms
- Process Vessels
  - Source Locations
    - Cleanroom Shoe Cover
    - Fermentor
    - Process Vessels
    - ✓ The Source was a Raw Material

Bacillus Testing



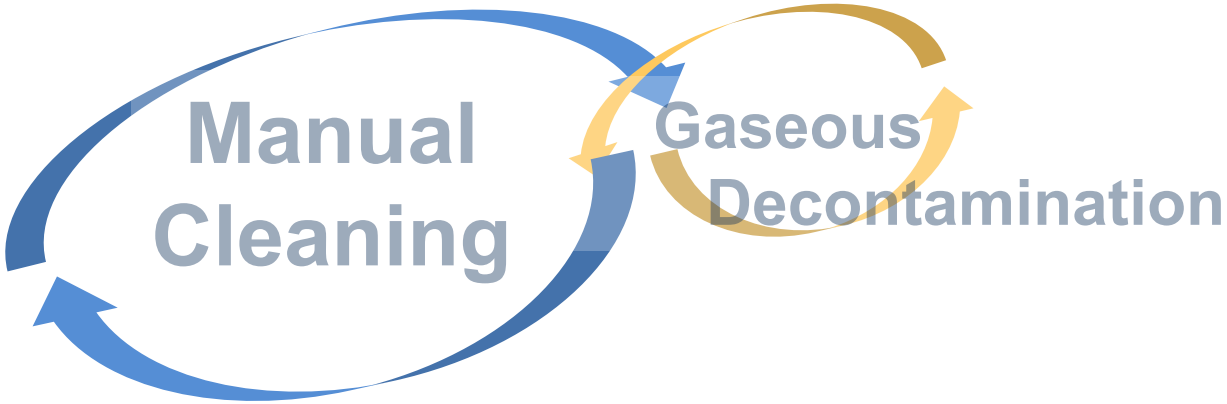
Spore testing results



Sporicidal Application

- H2O2/PAA Sporicides
- Cart Wheels
- Items entering the cleanroom

Gaseous Decontamination



Complementary approaches which form a highly-effective solution to manage bioburden in critical environments

Copyright © 2014 STERIS Corporation. All Rights Reserved. CONFIDENTIAL and PROPRIETARY to STERIS Corporation

Gaseous Decontamination Methods

STERIS
Life Sciences

DECONTAMINATION METHOD	DELIVERY MEDIUM	PERMISSIBLE EXPOSURE LIMIT*	HUMAN CARCINOGEN	EFFICACY	CYCLE TIME (2500FT^3)	MATERIAL COMPATIBILITY	REPEATABILITY (VALIDATION)
VHP	Vapor	1.0 ppm	No	Good	< 4 hrs	Good	Good
Hydrogen Peroxide (e.g., fogging, ionization, micro-condensation)	Hybrid	1.0 ppm	No	Good	4-8 hrs	Variable	Moderate
Chlorine Dioxide	Gas	0.1 ppm	No	Good	< 4 hrs	Moderate	Good
Formaldehyde	Gas	0.75 ppm	Yes	Good	> 8 hrs	Good	Good

* Values represent OSHA permissible exposure limit (PEL) for 8-hour time weighted average (TWA) exposure.

** Visible soils must be properly cleaned before VHP® application.

Why Use VHP?

STERIS
Life Sciences

- ✓ Efficacy (Broad spectrum sterilant)
- ✓ Consistency & Distribution
  - ➢ Reach difficult to access surfaces
  - ➢ Passes through HEPA filters
  - ➢ Kills airborne and surface microbes
- ✓ Excellent Material Compatibility
  - ➢ Electronics
  - ➢ Metals and common polymers
- ✓ Speed
  - ➢ Minimal labor required
  - ➢ Easy to validate (24-hr BI)
- ✓ Green Technology
  - ➢ Low toxicity
  - ➢ No residues
  - ➢ EPA approved

VHP Process Validation

- Biological Indicators
  - Geobacillus stearothermophilus (Strains 7953 or 12980)
  - 6-log for Biodecontamination / Sterilization
- Environmental Monitoring
- Swabbing

** G. stearothermophilus has been proven to be the most resistant organism to VH_2O_2.*

Vaporized Hydrogen Peroxide - Limitations

STERIS Life Sciences

- Limitations
  - Cannot be used while areas are occupied
  - Higher application cost than liquid chemistries
    - 3rd Party Service
    - Capital Equipment and training investment
  - “Lazy” gas needs distribution assistance in large space

Typical Decon Applications

STERIS
Life Sciences

Anything from a biosafety cabinet to a complete facility…

Laboratory Equipment
- Microbiological Safety Cabinets
- Transfer Chambers
- Isolators
- Incubators
- Centrifuges

Complete Facilities
- Clean-rooms
- Corridors
- Laboratories
- Containment facilities
- Changing Areas
- Prep Areas
- Analytical Work areas
- Offices
- Lockers
- Air Showers
- Wash / Toilet areas
- Ductwork
- Filters (HEPA)
- Storage Areas
- Service Areas
- Electrical Cabinets

When to Decontaminate

STERIS Life Sciences

- Proactive Basis
  - After shutdown or production change (e.g. Pharma)
  - Before shutdown or equipment service (e.g. BSL-3)
  - Bioburden reduction
- Elimination of Known Contamination (Event Response)
- Commissioning / Decommissioning
  - Facility
  - Equipment

VHP Process Development for Spaceship Applications

STERIS Life Sciences

Authors: Naresh Rohatgi (NASA JPL) & STERIS Strategic Technology Enterprises

Publication: 04ICES-113

Publication Date: 2004

- NASA Planetary Protection Office's microbial reduction requirements for all Mars *in situ* life detection missions may require entire spacecraft decontamination.
- Electronics not compatible with approved dry heat methods
- STERIS designed and constructed a high vacuum (~one torr) Biological Indicator Evaluator Resistometer (BIER) vessel to generate hydrogen peroxide lethality data.
- VHP process provided an effective, rapid, safe, and low temperature means for decontaminating spores, mycobacteria, fungi, viruses, and other microorganisms
- VHP process has innocuous residuals as it decomposes to water vapor and oxygen\
- Results under Implementation Plan for Jet Propulsion Laboratory, RG-563852 "Generation of Lethality Data on Vapor Phase Hydrogen Peroxide."

VHP Certification from NASA Interplanetary Protection

Authors: Chen, Fei; Chung, Shirley; Barengoltz, Jack

Affiliation: AA(Jet Propulsion Laboratory, California Institute of Technology), AB(JPL), AC(Private Individual)

Publication: 38th COSPAR Scientific Assembly,July 2010, in Bremen, Germany, p.4

Publication Date: 00/2010

- Flight system must deposit minimal bioload on planets
- Dry heat sterilization (only previously approved method) not suitable for electronics
- Validated using VHP "hardy" strains that were isolated from cleanrooms and environmental populations collected from spacecraft relevant areas.
- Material compatibility discussed

Case Study: Construction Event at Biotech Site

STERIS Life Sciences

- ❑ Worst Case Events
- ❑ 9X Clean [1X Sporicide + 2X Phenolic repeated on days 1,2,3]
- ❑ Fogging
- ❑ VHP®
- ❑ Triple Clean
  - ✓ Defined 3X Disinfectants and Sporicide
  - ✓ EM frequency (Static and Dynamic)
  - ✓ Release of the room

Triple Clean in a Cleanroom

Sample	Action Limit	Pre Triple Clean	Post Triple Clean
RODAC	2 cfu/plate	3 cfu/plate	<1 cfu/plate
RODAC	2 cfu/plate	31 cfu/plate	<1 cfu/plate
RODAC	2 cfu/plate	3 cfu/plate	<1 cfu/plate

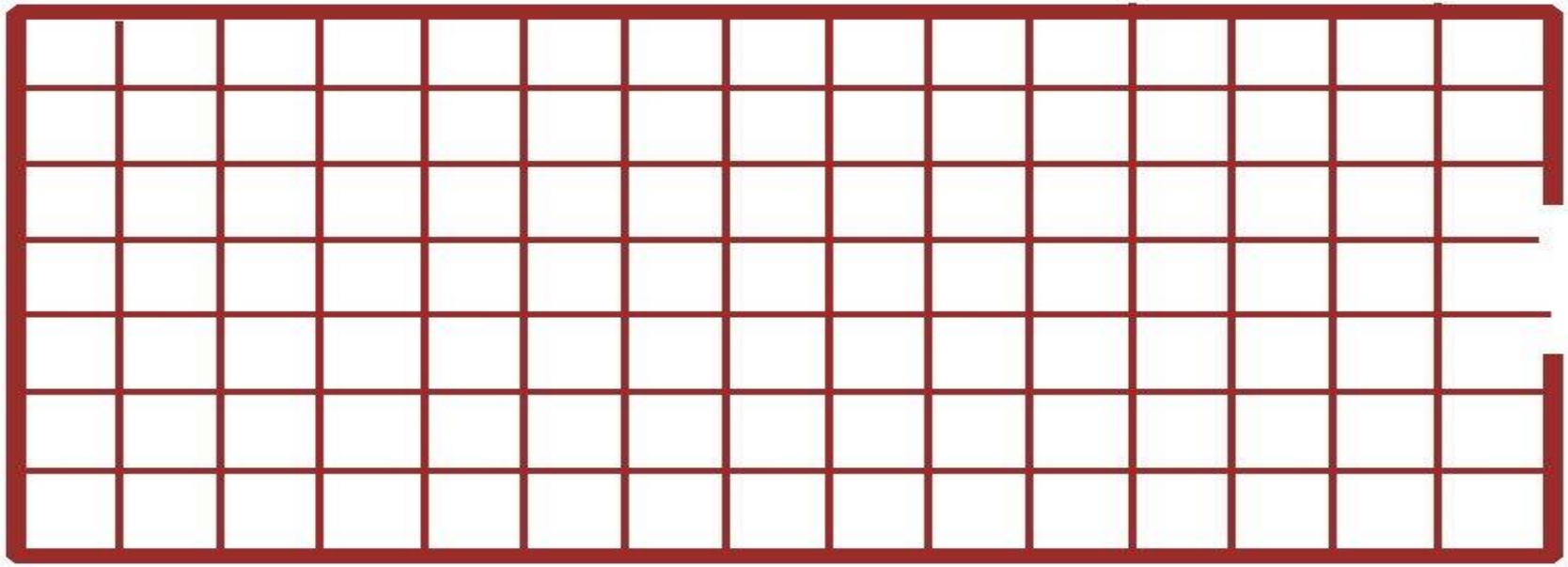
Results from an ISO-8 Cleanroom (554ft^2 room)

In Situ Data-Case Study

Room	Media Type	Action Limits	Pre-Sanitization[a]	Range (#cfu/unit)[b]	Post-Sanitization[a]	Range (#cfu/unit)[b]
#1	Biotest	>2.5 cfu/ft^3	3 of 4	0.3[d]	0 of 4	0
	RODAC	>2 cfu/plate	2 of 8	0 to 1	0 of 8	0
	Settling	>2 cfu/plate	0 of 4	0	0 of 4	0
	Swabs	>2 positive	0 of 4	N/A[c]	0 of 4	N/A[c]
#2	Biotest	>2.5 cfu/ft^3	1 of 4	0.04[d]	0 of 4	0
	RODAC	>2 cfu/plate	2 of 9	0 to 1	0 of 9	0
	Settling	>2 cfu/plate	0 of 4	0	1 of 4	0 to 1
	Swabs	>2 positive	1 of 7	N/A[c]	0 of 7	N/A[c]

Cleaning and Disinfection Efficacy

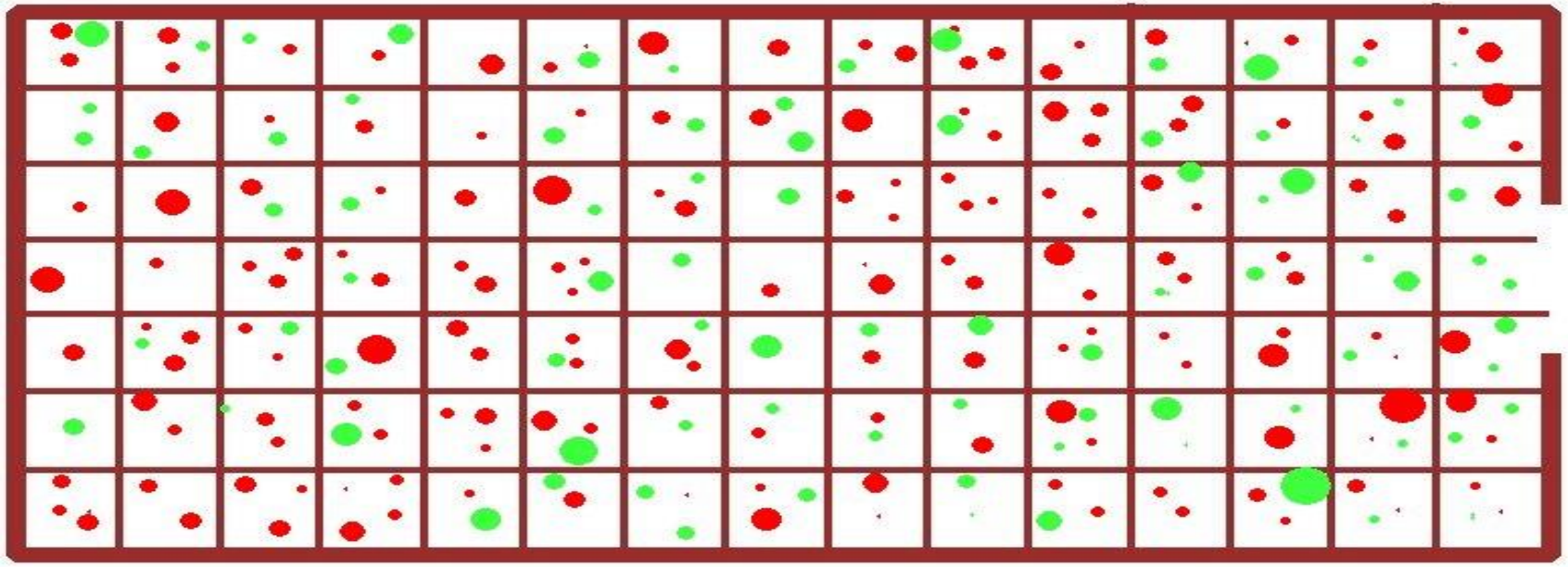
STERIS
Life Sciences



Time 0

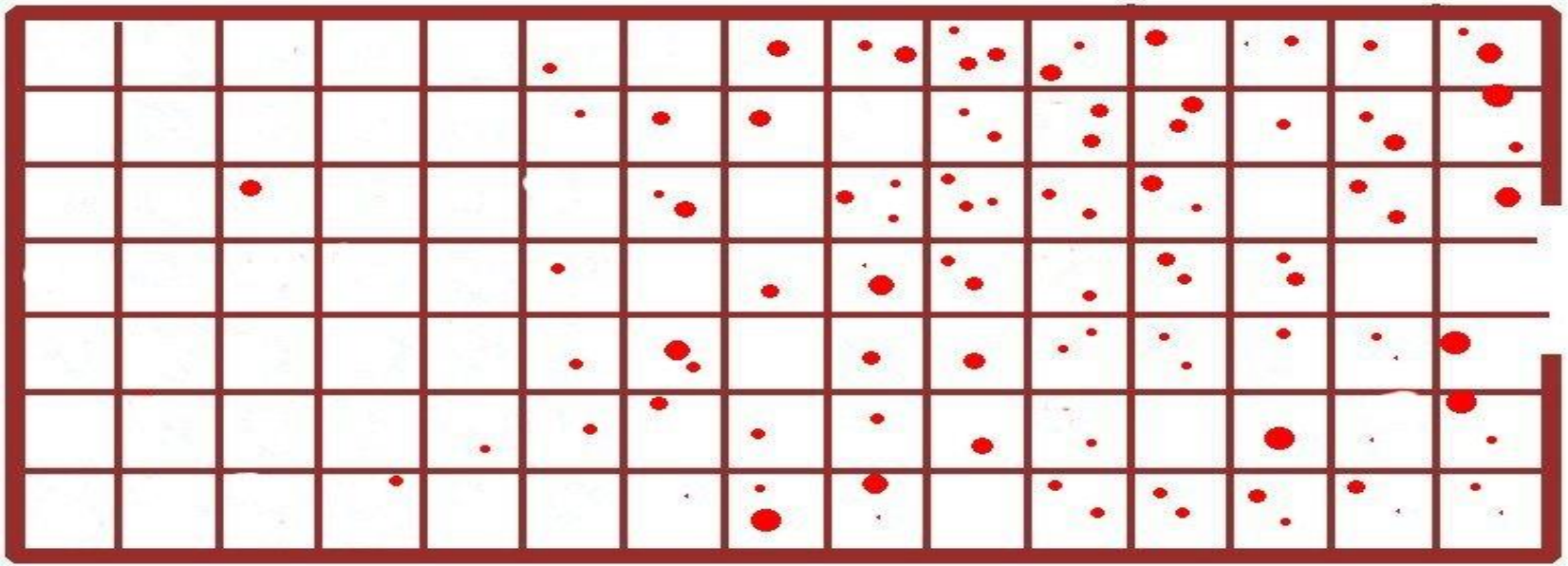
STERIS
Life Sciences

Red = Spore formers Green = Other

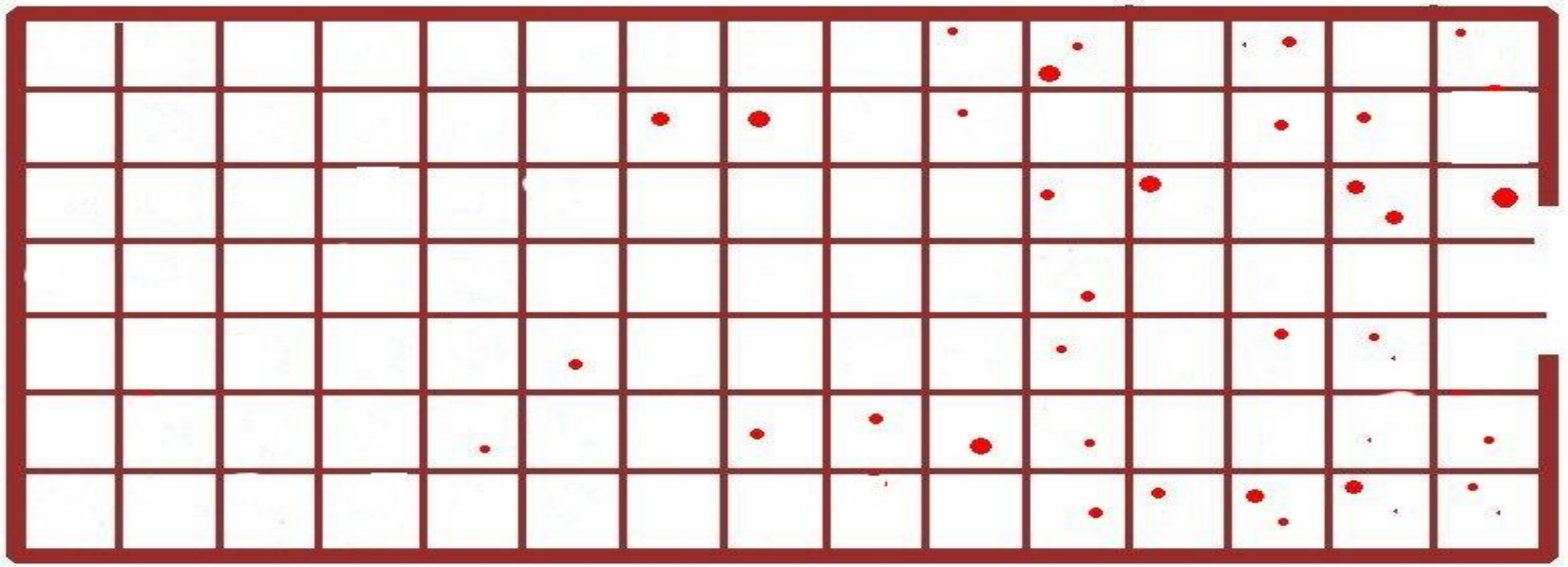


After 1X Cleaning - NO Sporicide

STERIS
Life Sciences

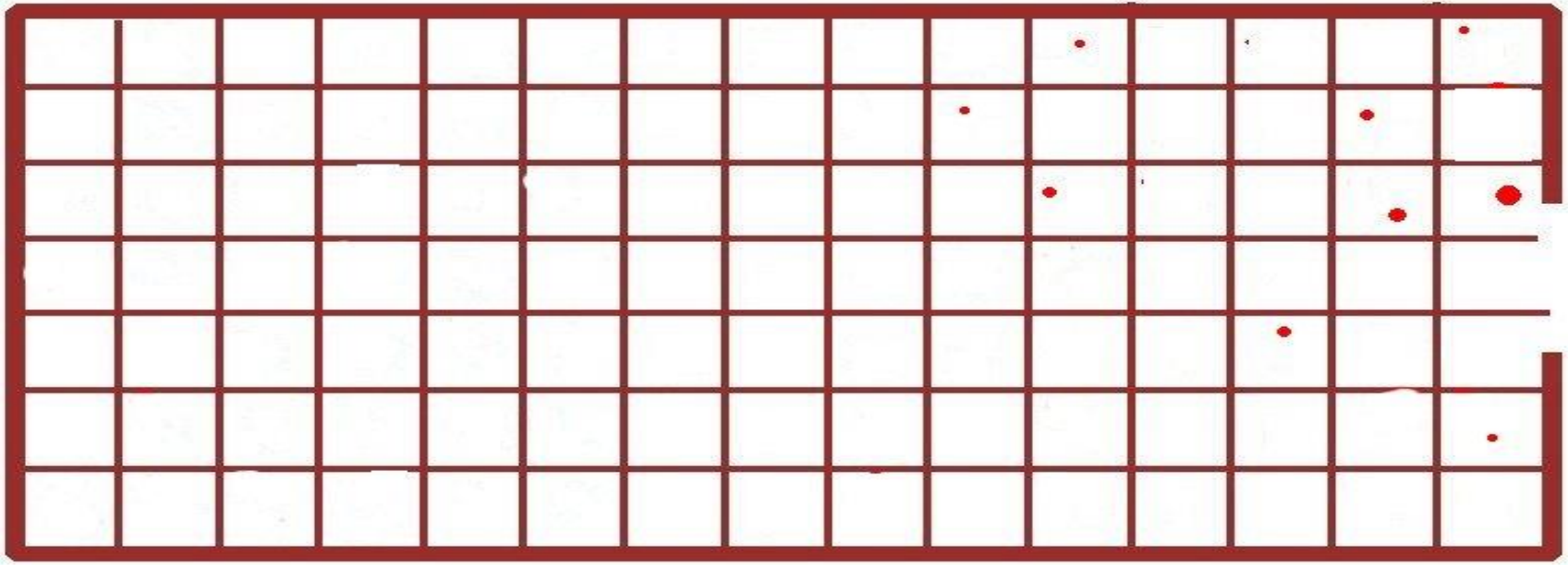


After 2X Cleaning – NO Sporicide

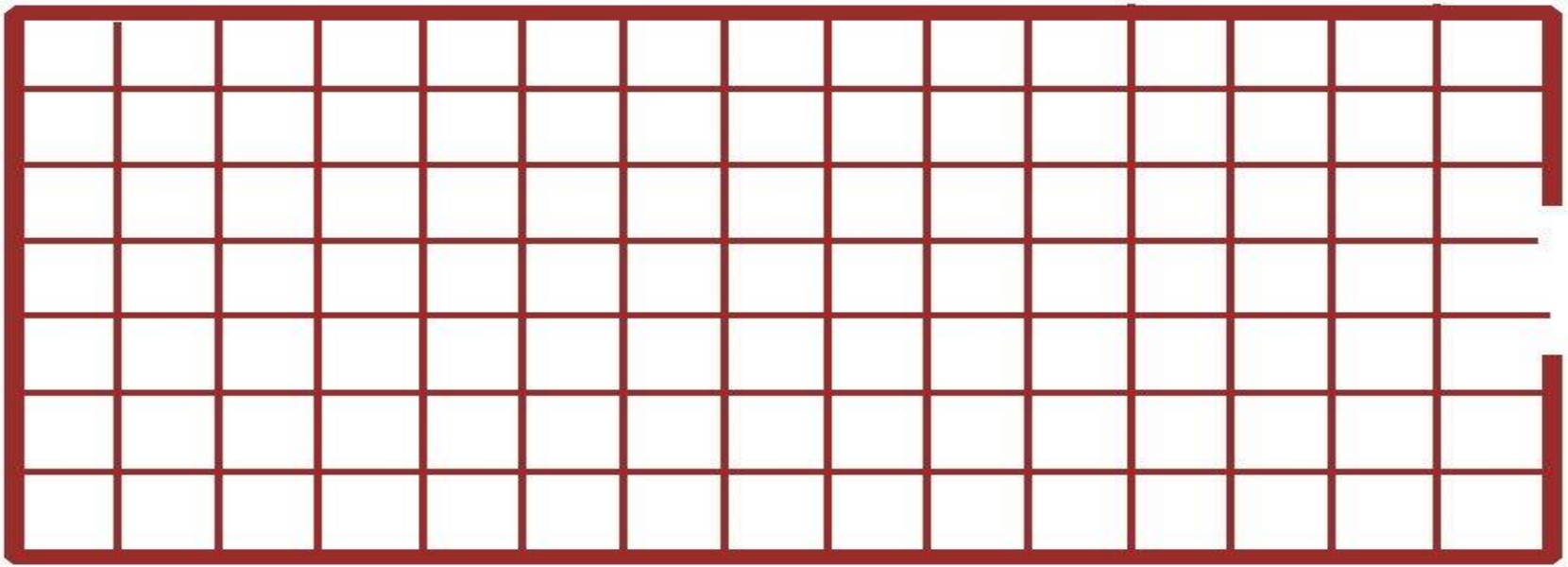


After 3X Cleaning - No Sporicide

STERIS
Life Sciences



After Sporicide



Summary

- Bioburden in Cleanrooms
  - Operator Contamination
  - Fungal Spore Contamination
  - Bacterial Spore Contamination
- Bacterial Spore Morphology and Efficacy Testing
- *In situ* testing case study

Thank You for Your Attendance!

STERIS
Life Sciences

jim_polarine@steris.com
larry_zanko@steris.com